Neurotrophins in Skin Biology and Pathology

Vladimir A. Botchkarev[1], Mina Yaar[1], Eva M.J. Peters[2], Siba P. Raychaudhuri[3], Natalia V. Botchkareva[4], Alessandra Marconi[5], Smriti K. Raychaudhuri[3], Ralf Paus[6] and Carlo Pincelli[5]

Neurotrophins (NTs) belong to a family of growth factors, which control the development, maintenance, and apoptotic death of neurons and also fulfill multiple regulatory functions outside the nervous system. Biological effects induced by NTs strongly depend on the pattern of NT receptor/co-receptors expression in target cells, as well as on the set of intracellular adaptor molecules that link NT signalling to distinct biochemical pathways. In this review, we summarize data on the molecular mechanisms underlying the involvement of NTs in the control of non-neuronal functions in normal skin (e.g. keratinocyte proliferation, melanocyte development and apoptosis, hair growth). We also review the data on the role for NTs and their receptors in a number of pathological skin conditions (stress-induced hair loss, psoriasis, atopic dermatitis). Although additional efforts are required to fully understand mechanisms underlying the involvement of NTs and their receptors in controlling functions of normal and pathologically altered skin cells, substantial evidence suggests that modulation of NT signalling by NTs receptor agonists/antagonists may be developed as intervention modalities in distinct skin and hair growth pathologies.

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Introduction

The common ectodermal origin of nervous system and skin epithelium suggests that growth factors regulating the development and function of neurons are also involved in the control of skin homeostasis and remodelling (Paus *et al.*, 1997; Pincelli and Yaar, 1997; Yaar, 1999; Botchkarev *et al.*, 2004). In this context, neurotrophins (NTs) are particularly important and fascinating study objects, as – as target-derived growth factors – they play an essential role in neuronal development, survival, outgrowth, and in the selective elimination of distinct neuronal sub-populations by apoptosis (Teng and Hempstead, 2004). Furthermore, it is well accepted now that, besides their involvement in the control of neuronal development, NTs together with other neurotrophic factors (e.g. ciliary-neurotrophic factor, glial cell line-derived neurotrophic factor) fulfill multiple non-neuronal functions and regulate cell proliferation, differentiation, apoptosis, and tissue remodelling outside of the nervous system (Sariola, 2001; Aloe, 2004).

During the last decade, substantial progress has been achieved in defining the roles for NTs and their receptors in the control of skin homeostasis and hair growth (for a review, see Paus *et al.*, 1997; Pincelli and Yaar, 1997; Yaar, 1999; Bonini *et al.*, 2003; Botchkarev *et al.*, 2004). Here, we review the molecular mechanisms underlying the involvement of NTs in the control of non-neuronal functions in normal skin, as well as the role for NTs in a number of pathological skin conditions, focusing on stress-induced hair loss, psoriasis, and atopic dermatitis. We also provide evidence that modulation of NT signalling by NT receptor agonists/antagonists may be used as a novel approach for managing distinct skin and hair growth abnormalities.

NTs and their receptors

The NT family consists of four structurally and functionally related proteins: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4). All four members of the NT family are synthesized as precursors, which are cleaved by intracellular proteases to release the C-terminal mature proteins (reviewed in Teng and Hempstead, 2004). Mature NT proteins are approximately 13 kDa in size, share about 50% of amino-acid sequence homology, and exert their biological effects as dimers interacting with specific receptors (for a review, see Huang and Reichardt, 2001). High-affinity receptors for NTs belong to the tyrosine kinase family: tyrosine kinase receptor A (TrkA) is the high-affinity

[1]*Department of Dermatology, Boston University School of Medicine, Boston, Massachusetts, USA;* [2]*Biomedical Research Centre, University Medicine Charite, Berlin, Germany;* [3]*Department of Genetics, Stanford University School of Medicine, Stanford, California, USA;* [4]*Gillette Advanced Technology Centre, The Gillette Company, Needham, Massachusetts, USA;* [5]*Institute of Dermatology, University of Modena and Reggio Emilia, Modena, Italy and* [6]*Department of Dermatology, University of Luebeck, Luebeck, Germany*

Correspondence: Dr Vladimir A. Botchkarev, Department of Dermatology, Boston University School of Medicine, 609 Albany Street, Boston, Massachusetts 02118, USA. E-mail: vladbotc@bu.edu

Abbreviations: BDNF, brain-derived neurotrophic factor; FGF, fibroblast growth factor; HF, hair follicle; NGF, nerve growth factor; NT-3/NT-4, neurotrophins 3/4; p75NTR, p75 kDa neurotrophin receptor; TrkA/B/C, tyrosine kinase receptors A/B/C

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receptor for NGF, tyrosine kinase receptor B (TrkB) is the high-affinity receptor for BDNF and NT-4, and tyrosine kinase receptor C (TrkC) is the high-affinity receptor for NT-3 (Segal, 2003). However, NT-3 may also bind – with low affinity – to TrkA and TrkB receptors. All four NTs interact with the low-affinity p75 kDa NT receptor (p75NTR), which is a member of the tumor necrosis factor family of receptors containing the cytoplasmic ''death'' domain, involved in mediating a number of responses independently or in association with Trk receptors (for a review, see Dechant and Barde, 2002; Roux and Barker, 2002; Teng and Hempstead, 2004).

By interacting with Trk receptors and/or p75NTR, NTs induce a variety of biological reactions in neurons and non-neuronal cells and control proliferation, differentiation, and survival (Table 1). The signals promoting survival or differentiation are generated by NT interaction with Trk receptors and require receptor dimerization, autophosphorylation, and the subsequent involvement of a number of adaptor molecules coupling Trk receptors to the distinct intracellular signal transduction pathways (Segal, 2003). NTs modulate synaptic transmission via Trk-associated regulation of intracellular Ca^{2+}, promote survival via phosphorylation and inactivation of several proapoptotic substrates including Bad, as well as promote differentiation via activation of the Ras/Raf/ERK kinse/mitogen-activated protein kinase cascade (reviewed in Segal, 2003).

p75NTR fulfils distinct functions depending on whether it is co-expressed with Trk receptors and/or selected other growth factor receptors (sortilin, Nogo receptor complex), or whether it is expressed alone (Table 1). The co-expression of p75NTR with Trk receptors increases high-affinity NT binding, enhances Trk ability to discriminate a preferred ligand from the other NTs, and promotes survival effects of the NTs (Dechant and Barde, 2002; Roux and Barker, 2002; Teng and Hempstead, 2004). When p75NTR is co-expressed with sortilin (a non-G-protein-coupled neurotensin receptor), NT precursor proteins (pro-NTs) interacting with p75NTR–sortilin complex induce apoptotic death (reviewed in Nykjaer *et al.*, 2005). In case of co-expression of p75NTR with the Nogo receptor complex, Nogo induces growth inhibition (reviewed in Teng and Hempstead, 2004).

When p75NTR is expressed alone on the cell surface, mature NT peptides or selected non-NT ligands (beta-amyloid or a fragment of the prion protein) are capable of inducing apoptosis or promote survival depending on the intracellular adaptor molecules present in target cells (Yaar *et al.*, 2002; Teng and Hempstead, 2004). Apoptotic signalling via p75NTR requires the presence of intracellular adaptor molecules (NT receptor-interacting factors 1 and 2, NT receptor-interacting MAGE homolog, and NT-associated death executor) that link p75NTR signalling with the JNK-p53-Bax proapoptotic pathway (Table 1). However, signalling through p75NTR expressed alone – besides inducing apoptosis – may also promote cell survival. Intracellular adaptor molecules interacting with the C-terminus of p75NTR (TNF receptor-associated factor 6, Fas-associated phosphatase-1, and receptor interacting protein-2) link p75NTR with the NF-κB pathway and can thus promote survival (Roux and Barker, 2002). However, mechanisms involved in the controlling the expression and preferential engagement of adaptor molecules in distinct cell types remain to be clarified. These growing, ever more complex insights into NT-mediated signalling must be kept in mind when interpreting the data obtained so far on the effects of NTs in distinct skin cell populations.

Non-neuronal targets for NTs in the skin

In mice, NTs are expressed very early during embryonic development (E9.5–E10.5) in both the skin epithelium and the cutaneous mesenchyme (reviewed in Ernfors *et al.*, 1994). The onset of NT expression in embryonic

Table 1. Biochemical components of the distinct signalling pathways activated by NTs and their receptors[1]

Ligand(s)	Receptor(s)	Co-receptor(s)	Intracellular adaptor molecule(s)	Intracellular signaling pathway(s)	Biological effect(s)
NGF, BDNF, NT-3, NT-4	Trk A, Trk B, or TrkC	p75NTR	Shc, Grb2, Gab-1	PI3K/PKB/AKT	Survival
NGF, BDNF, NT-3, NT-4	Trk A, Trk B, or TrkC	p75NTR or none	Shc, Grb2, SOS, ARMS, Ras, Raf	Ras/Raf/MEK/MAPK	Differentiation
NGF, BDNF, NT-3, NT-4	Trk A, Trk B, or TrkC	p75NTR	PLC-γ	IP3/DAG	Ca^{2+} release
NGF, BDNF, NT-3, NT-4	p75NTR	—	TRAF6, FAP-1, RIP-2	NF-κB	Survival
NGF, BDNF, NT-3, NT-4	p75NTR	—	NRIF1/2, NRAGE, NADE	Jnk, p53, Bax, caspases 9/6/3	Apoptosis
Pro-NGF	p75NTR	Sortilin	ND	ND	Apoptosis
Nogo	p75NTR	Nogo receptor, Lingo-1	ND	ND	Growth inhibition

[1]For details, see the corresponding review articles (Dechant and Barde, 2002; Roux and Barker, 2002; Segal, 2003; Teng and Hempstead, 2004; Nykjaer *et al.*, 2005).
JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MEK, ERK kinse; NADE, neurotrophin-associated death executor; NRAGE, neurotrophin receptor interacting MAGE homolog; NRIF1/2, neurotrophin receptor-interacting factors 1 and 2; ND, not determined; PKB, protein kinase B; PI3K, phosphatidylinositol 3′-kinase; PLC, phospholipase C.

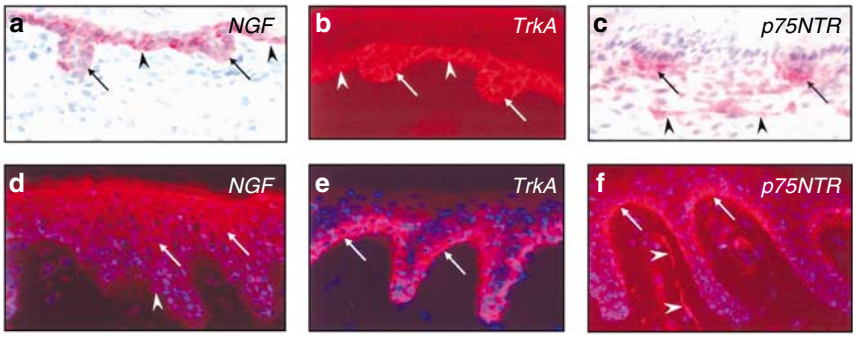

Figure 1. Expression of NTs and their receptors in mouse and human skin. Cryostat sections of mouse and human skin were immunostained with antisera to NGF, TrkA, and p75NTR. (**a–c**) Mouse embryonic skin. (**a**) NGF and (**b**) TrkA expression in basal layer of epidermis (arrowheads) and hair placodes (arrows) in embryonic mouse skin. (**c**) p75NTR expression in the mesenchyme of developing HFs (arrows) and in Schwann cells (arrowheads) in embryonic mouse skin. (**d–f**) Human adult skin. (**d**) NGF is expressed in suprabasal keratinocytes (arrows) and in single cells of the basal epidermal layer (arrowhead). (**e**) TrkA expression in basal epidermal keratinoicytes (arrows). (**f**) p75NTR expression in basal epidermal keratinocytes with predominance in the inter-rete areas (arrows) and in cutaneous nerve fibers/Schwann cells (arrowheads).

skin coincides with the time point of appearance of K5 and K14 in the epidermis (E9.5), whereas maximal NT synthesis coincides with the beginning of vibrissa development in facial skin (E12.5), and with the initiation of tylotrich hair follicle (HF) induction in dorsal murine skin (E14.5) (Ernfors *et al.*, 1994). This suggests the hypothesis that NTs fulfill multiple non-neurotrophic functions during skin development.

In postnatal skin, NTs and their receptors are differentially distributed in distinct cell populations (Figure 1a–c). Basal epidermal keratinocytes in humans and mice express NGF and NT-4 (Di Marco *et al.*, 1991, 1993; Pincelli *et al.*, 1994, 1997; Botchkarev *et al.*, 1999). NGF, NT-3, and BDNF are produced by fibroblasts *in vitro*, and NGF stimulates fibroblast migration (reviewed in Yaar, 1999). *In situ*, BDNF and NT-3 are expressed in cutaneous nerve fibers and myocytes of the arrector pili and panniculus carnosus muscles (Botchkarev *et al.*, 1999). All four NT receptors (TrkA, TrkB, TrkC, and p75NTR) have been detected on human epidermal keratinocytes (Bronzetti *et al.*, 1996; Grewe *et al.*, 2000). In murine skin, only TrkA and TrkB isoforms are seen in epidermal keratinocytes, whereas TrkC and p75NTR are expressed in cutaneous nerves and in the HF (Botchkarev *et al.*, 1999).

NTs and epidermal keratinocytes

Work over the past 10 years has indicated that NTs possess a range of functions outside the nervous system (Sariola, 2001; Bonini *et al.*, 2003; Aloe, 2004) and can be considered as growth factors in epithelial tissue homeostasis. It was demonstrated that normal human keratinocytes synthesize and secrete biologically active NGF (Di Marco *et al.*, 1991; Yaar *et al.*, 1991). In human skin, NGF is released in increasing amounts by proliferating keratinocytes, whereas secretion ends in more differentiated cells (Pincelli *et al.*, 1994; Stefanato *et al.*, 2003; Figure 1d). Both exogenous and endogenous NGF are capable of inducing keratinocyte proliferation (Di Marco *et al.*, 1993). On the other hand, in the presence of their normal mesenchymal environment, exogenous NGF can indeed either stimulate or inhibit murine epidermal and HF keratinocyte proliferation *in situ*, depending on whether the keratinocytes are in a state of relative quiescence or are already maximally proliferating (Paus *et al.*, 1994). The proliferative effects of autocrine NGF on human keratinocytes are also confirmed by the use of the natural alkaloid K252a, an inhibitor of TrkA phosphorylation. Indeed, K252 blocks keratinocyte proliferation, in the absence of exogenous NGF (Pincelli *et al.*, 1994). Moreover, human keratinocytes transfected with NGF proliferate to a significantly greater extent than mock-transfected cells (Marconi *et al.*, 1999).

Keratinocytes express and release NTs, other than NGF (Botchkarev *et al.*, 1999; Marconi *et al.*, 2003), and BDNF, NT-3, and NT-4 stimulate murine epidermal keratinocyte proliferation *in situ* (Botchkarev *et al.*, 1999). NGF is secreted at highest levels as compared to the other NTs, whereas NT-3 and NGF upregulate each other's secretion in human keratinocytes. NGF expression is downregulated by UVB irradiation (Marconi *et al.*, 1999; Stefanato *et al.*, 2003), whereas NT-3 release is augmented by UVA.

At the skin level, TrkA and TrkC mediate NGF- and NT-3-induced keratinocyte proliferation, respectively. Indeed, keratinocytes overexpressing TrkA proliferate significantly better than controls (Pincelli, 2000), and increasing concentrations of anti-NT-3 antibody inhibit keratinocyte proliferation (Marconi *et al.*, 2003). In mouse skin, epidermal keratinocytes express TrkA and TrkB, and all NTs are capable of stimulating their proliferation in *ex vivo*-cultured skin explants (Paus *et al.*, 1994; Botchkarev *et al.*, 1999).

Apoptosis plays a fundamental role in epidermal homeostasis by counterbalancing cell proliferation, and apoptotic cells are consistently present in normal human epidermis (Pincelli *et al.*, 1997; Wehrli *et al.*, 2000). *In vitro,* NGF, but not the other NTs, can rescue human epidermal keratinocytes from spontaneous and UVB-induced apoptosis via TrkA (Pincelli *et al.*, 1997; Marconi *et al.*, 1999, 2003). Although UVB downregulates NGF and TrkA in human keratinocytes, NGF-overexpressing keratinocytes are protected from UVB-induced apoptosis (Marconi *et al.*, 1999).

NGF protects keratinocytes from cell death via the Bcl-2 family of apoptosis inhibitors. Indeed, K252a fails to induce apoptosis in keratinocytes overexpressing Bcl-2, and UVB causes a decrease in Bcl-2 and Bcl-xL expression in mock-transfected keratinocytes, but not in NGF-overexpressing cells. NGF prevents the cleavage of the enzyme poly(ADP-ribose) polymerase, a substrate for caspases, that is induced in human keratinocytes by UVB

(Marconi *et al*., 1999). These results are consistent with a model whereby autocrine NGF protects human keratinocytes from apoptosis through its high-affinity receptor TrkA by maintaining constant levels of Bcl-2 and Bcl-xL, which in turn block caspase activation.

The above-mentioned data clearly show that NTs mediate proliferative and survival signals in epidermal keratinocytes through their high-affinity Trk receptors. Still, the role of the low-affinity p75NTR in NGF signalling in keratinocytes remains to be clarifed. Although TrkA is evenly distributed in the basal keratinocyte layer, p75NTR is expressed in basal keratinocytes with an irregular pattern (Figure 1e and f). As human keratinocytes lack functional TrkB (Marconi *et al*., 2003), BDNF and NT-4 obviously signal through p75NTR in these cells. Indeed, BDNF and NT-4 induce apoptosis in cultured human keratinocytes (Atzei *et al*., manuscript in preparation). This is in agreement with the observation of a similar function of p75NTR in the catagen phase of the hair cycle (Botchkarev *et al*., 2000). Therefore, a balance between the low- and the high-affinity NT receptors exists in keratinocytes. However, the exact stimuli and conditions whereby NGF and other NTs signal life or death in keratinocytes are yet to be defined. Also, it remains to be determined whether NTs and their receptors could play a role in the development of non-melanoma skin cancers by stimulating proliferation and inhibiting apoptosis, in a manner similar to what has been shown for prostate (Krygier and Djakiew, 2001) and breast neoplasia (reviewed in Nakagarawa, 2001).

NTs and melanocytes

During skin development, neural crest-derived melanoblasts migrate into the skin and differentiate into melanocytes, which populate the basal layer of the epidermis and the HFs. Together with other paracrine signalling molecules (fibroblast growth factor (FGF), bone morphogenetic proteins, noelin-1, stem cell factor, hepatocyte growth factor, endothelins), NTs play an important role in the control of melanoblast migration, viability, and differentiation (reviewed in Pincelli and Yaar, 1997; Yaar, 1999).

Normal human melanocytes also express p75NTR and its expression level is upregulated by a variety of stimuli including UV irradiation (Peacocke *et al*., 1988). Keratinocyte-derived NGF, which expression is also upregulated by UV irradiation (Di Marco *et al*., 1991; Yaar *et al*., 1991), may influence epidermal melanocytes in a paracrine manner. *In vitro*, NGF is chemotactic for melanocytes and stimulates melanocyte dendrite formation (Yaar *et al*., 1991). Although under optimal basal culture conditions, there is no effect of NGF on melanocyte cell yields or melanogenesis, both NGF and NT-3, the latter expressed by dermal fibroblasts (Yaar *et al*., 1994), increase melanocyte survival when the cells are maintained in medium depleted of growth factors (Yaar *et al*., 1994; Zhai *et al*., 1996).

Interestingly, phorbol 12-tetra decanoate 13 acetate, a strong activator of protein kinase C, upregulates the expression of p75NTR and induces the expression of TrkA in melanocytes (Yaar *et al*., 1994). Although the exact mechanism that regulates phorbol 12-tetra decanoate 13 acetate-induced p75NTR and TrkA upregulation is not known, phorbol 12-tetra decanoate 13 acetate is recognized to have a striking effect also on melanocyte dendricity. It is possible that this differentiated morphology of melanocytes is part of an integrated complex of differentiated functions that includes induction of receptors to NGF. In contrast with TrkA expression that requires induction, melanocytes constitutively express TrkC, albeit the expression is likely to be low as it was detected by the sensitive reverse transcriptase-PCR methodology (Yaar *et al*., 1994). Also, in contrast with TrkA expression, TrkC expression is decreased after phorbol 12-tetra decanoate 13 acetate, suggesting that although melanocytes can bind both NGF and NT-3, different signals that preferentially induce a specific high-affinity receptor determine which NT would exert its effect. Thus, NGF and NT-3 effect in melanocytes may be influenced by outside signals through modulations of their high-affinity receptor expression.

Indeed, using UV-irradiated cultured melanocytes and human melanoma cells, NGF supplementation enhances cell survival, markedly reduces apoptotic cell death, and increases the level of the antiapoptotic Bcl-2 protein which is expressed strongly by melanocytes *in vivo* even in the absence of UV irradiation (Zhai *et al*., 1996; Stefanato *et al*., 2003). The data suggest that NGF, which is constitutively produced by neighboring epidermal keratinocytes, may preserve the population of cutaneous melanocytes that would otherwise be depleted by sun-exposure. In contrast, NT-3, which is strongly expressed by non-proliferating fibroblasts (Yaar *et al*., 1994), like those in the dermal compartment of non-damaged human skin, could help in melanocyte maintenance during steady-state conditions.

NTs in the control of HF development and cycling

The HF is an unusually densely innervated peripheral organ, whose development is governed by epitheliomesenchymal interactions between epidermal keratinocytes committed to HF-specific differentiation and a cluster of dermal cells that form the follicular papilla (Schmidt-Ullrich and Paus, 2005). In postnatal life, HF transits through its lifelong cycle of growth (anagen) to apoptosis-driven regression (catagen) and finally relative resting (telogen) (Stenn and Paus, 2001). As the role for NTs and their receptors in the regulation of HF development and cycling were recently reviewed in details elsewhere (Botchkarev *et al*., 2004), we will only briefly summarize the most important aspects of NT involvement in hair growth control.

During skin development, intracutaneously generated NTs are not only required for appropriate skin innervation but also play an important role in the control of HF morphogenesis. This is evident from the observation that murine HFs show developmentally and spatiotemporally stringently controlled expression of NTs and their cognate receptors (TrkA, TrkB, TrkC, p75NTR; Figure 1a–c) (Botchkarev *et al*., 1998; Botchkareva *et al*., 1999, 2000). Furthermore, data obtained from

genetically engineered mice with constitutive overexpression or deletion of NGF, BDNF, and/or NT-3/TrkC revealed that NGF and NT-3 significantly accelerate HF morphogenesis, whereas BDNF do not show any significant effects (Botchkarev *et al.*, 1998, 2004; Botchkareva *et al.*, 2000).

In contrast to Trk receptor signalling, p75NTR that is expressed in the follicular papilla plays an *inhibitory* role during murine HF morphogenesis (Botchkareva *et al.*, 1999). Compared to age-matched wild-type animals, p75NTR knockout (−/−) mice showed a significant acceleration of HF development associated with increased expression of FGF-2 receptor (FGFR-2) that specifically binds to FGF7/KGF, in the dermal papilla and outer root sheath (Botchkareva *et al.*, 1999). As the acceleration of HF development in p75NTR knockout mice could be overcome by administration of FGF7-neutralizing antibody, this suggests that, in developing murine HF, p75NTR downregulates FGF7-signalling through FGFR-2 and thus acts as a ''brake'' on HF morphogenesis by limiting the effects of FGF7 on early steps of HF development (Botchkareva *et al.*, 1999).

During later HF cycling, follicular NTs and NT receptor expression display hair cycle-dependent fluctuations on the mRNA and protein level, which are mirrored by changes in nerve fiber density and neurotransmitter/neuropeptide content in the perifollicular neural networks (reviewed in Botchkarev *et al.*, 2004). During the murine hair cycle, steady-state levels of NGF and NT-3 proteins fluctuate differently: while NGF levels rise dramatically in early anagen skin, NT-3 protein was significantly upregulated during catagen. Also, the steady-state levels of NT-3, BDNF, and NT-4 mRNAs were significantly increased prior and during catagen onset (reviewed in Botchkarev *et al.*, 2004). Furthermore, neonatal transgenic mice overexpressing NGF, NT-3, or BDNF display accelerated catagen development, and BDNF-overexpressing mice have a significant shortening of hair length compared to the corresponding age-matched wild-type animals. Conversely, NT-3, BDNF, or NT-4 null mutants show catagen retardation (Botchkarev *et al.*, 2004).

The addition of NGF, NT-3, NT-4, and BDNF *in vitro* significantly stimulate catagen development in C57BL/6 murine skin organ cultures, whereas p75NTR antagonist cyclic decapeptide abrogates catagen-stimulatory effects of NTs and retards catagen *in situ* (Botchkarev *et al.*, 2000, 2004; Peters *et al.*, 2006). By using p75NTR knockout mice, we showed that catagen-stimulatory effects of NTs is mediated by their binding via p75NTR expressed in keratinocytes of the regressing outer root sheath (Botchkarev *et al.*, 2000).

In cultured human anagen HFs, NGF and BDNF suffice to induce premature entry into a catagen-like stage (Peters *et al.*, 2005, 2006), which can be antagonized by transforming growth factor-β2- or p75NTR-neutralizing antibodies. Interestingly, the newly discovered high-affinity ligand for p75NTR, pro-NGF, is present in the terminally differentiated inner root sheath of human anagen HFs and is upregulated in the same apoptosis-rich compartments as p75NTR during spontaneous catagen-like regression of human anagen HFs (Peters *et al.*, 2006), suggesting that both NGF and pro-NGF are involved in controlling apoptosis in human HF keratinocytes.

Taken together, these data suggest that the HF serves as both a prominent target and key peripheral source of NTs in the skin, and that appropriate NT receptor ligands could be used not only for treating unwanted hair loss (alopecia, effluvium) but also for the management of excessive, unwanted hair growth (hirsutism, hypertrichosis).

Immunomodulatory functions of NTs in the skin

Numerous publications suggest that NTs play an important role in regulating the activity of immune cells in normal skin and in a number of pathological conditions including wound healing, inflammation, psoriasis, and atopic dermatitis, as well as in the allergic, autoimmune, and stress-induced skin responses (reviewed in Ansel *et al.*, 1997; Legat *et al.*, 2002; Steinhoff *et al.*, 2003; see also below). In normal skin (see Figure 2), mast cells, endothelial cells, and macrophages express NGF, whereas TrkA receptor is expressed on mast cells and endothelial cells (Raychaudhuri and Raychaudhuri, 2004; Groneberg *et al.*, 2005; Raap and Kapp, 2005).

Similarly to other organs, NGF stimulates degranulation and cytokine release from skin mast cells and thereby promotes neurogenic inflammation (reviewed in Legat *et al.*, 2002; Steinhoff *et al.*, 2003). During inflammation, NGF stimulates tissue nociceptors and enhances inflammatory pain, as well as increases vascular permeability followed by tissue edema via stimulation of calcitonin gene-related peptide release from sensory nerve endings and via induction of mast cell

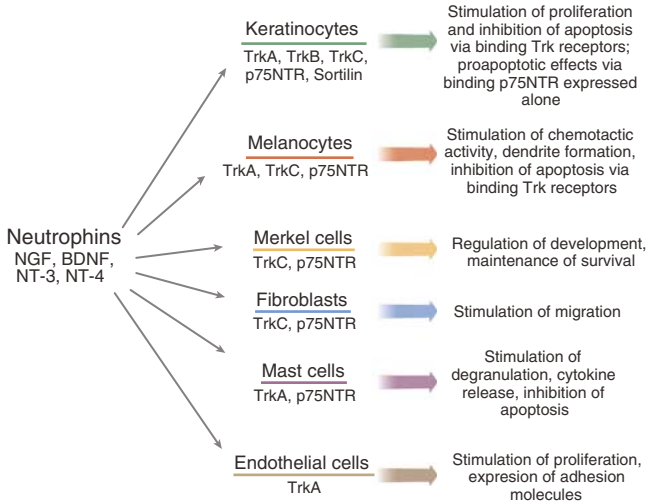

Figure 2. Scheme illustrating the effects of NTs on distinct cell populations in skin.

degranulation (Aloe, 2004). However, mast cell-derived NGF may also play a role in establishing the contacts between mast cells and sensory nerve fibers in normal skin, which frequency fluctuates significantly in a hair cycle-dependent manner (reviewed in Botchkarev *et al.*, 2004). Such contacts are a prerequisite of the increased mast cell degranulation observed upon stress-exposure (Peters *et al.*, 2005c) and increase in number under stress, which upstream of substance P (SP) may also be responsible for the increased SP-induced neurogenic inflammation observed upon stress-exposure (Peters *et al.*, 2004).

In addition to the regulation of these inert immune responses, NGF is involved in the activation, migration, and proliferation of endothelial cells, lymphocytes, and macrophages in the skin, as well as in the expression of adhesion molecules on endothelial cells, thus implicating its role in controlling the traffic of blood cells into the skin and in the promotion of a specific immune response and cytokine production with implications for chronic inflammatory, allergic, and autoimmune diseases (Raychaudhuri *et al.*, 2001; Raap and Kapp, 2005). However, although NTs are involved in the control of lymphocyte development and T-lymphocytes are capable of producing NGF and BDNF (for a review, see Vega *et al.*, 2003), only little is known about the expression of NTs and their receptors on distinct lymphocyte subpopulations in normal and diseased skin.

NTs in pathological skin conditions

Increasing evidence suggests that NTs are also involved in the pathogenesis of inflammatory skin diseases characterized either by cell loss (stress-induced alopecia) or hyperproliferation (inflammation, wound healing, psoriasis, atopic dermatitis) via neurogenic inflammatory processes or by inducing a misbalance in cytokine production and autoimmune responses. As the role of NTs in pathogenesis of the inflammatory response and wound healing has been reviewed elsewhere (Bonini *et al.*, 2003; Kawamoto and Matsuda, 2004), here we shall only sketch some recently emerging concepts on the role of NTs in stress-induced hair loss, atopic dermatitis, and psoriasis.

NTs in the HF response to psychoemotional stress. It has been shown that upon acute stress-exposure, NGF is released into the blood, for example, from submaxillary glands of male mice (cf. Alleva *et al.*, 1996) and perhaps other sources, making it a likely mediator of stress. The role of NGF as a local stress-response mediator involved in neurogenic inflammation was also recently established in a mouse model for perceived psychoemotional stress (Arck *et al.*, 2001; Peters *et al.*, 2004, Paus *et al.*, 2006). In this model, stress-induced increase in the numbers of apoptotic cells in the HF can be antagonized by NGF neutralization (Peters *et al.*, 2004), demonstrating an important role for NGF in stress-induced HF damage. Remarkably, NGF serves as a trigger that mediates a complex skin and HF responses to stress involving in addition neuropeptides released from sensory nerve fibers and mast cell mediators and cytokines (Arck *et al.*, 2001, 2003). Thus, NGF can promote outgrowth of substance P-positive nerve fibers in this model, for example, towards mast cells, thereby building up a network that allows for increased neurogenic inflammatory responses upon stress (Peters *et al.*, 2004). At the same time, NGF alone can stimulate mast cell degranulation and release of proinflammatory cytokines by itself (Groneberg *et al.*, 2005; Raap and Kapp, 2005). Although the roles for Trk and p75NTR in the control of this very complex pathobiological response remain to be elucidated, these data suggest that both Trk and p75NTR antagonists may be used for preventing stress-induced skin and hair growth abnormalities.

NTs and allergic skin responses. Allergic skin responses like those occurring in allergic contact dermatitis or atopic dermatitis depend on neurogenic inflammation, but even more on the production of certain proinflammatory cytokines (e.g. IL-4; reviewed in Raap and Kapp, 2005). Accordingly, NGF and p75NTR expression were shown to be increased on nerve fibers, Schwann cells, mast cells, eosinophils, and keratinocytes in the skin affected by allergic contact dermatitis, prurigo and atopic dermatitis, and in the serum of patients with atopic dermatitis (Groneberg *et al.*, 2005; Raap and Kapp, 2005). This increase in NGF may be responsible for the increased number of nerve fibers frequently observed in these skin conditions since NGF expression was found in a mouse model of atopic dermatitis, which is also characterized by increased SP+ nerve fibers in the skin (reviewed in Raap and Kapp, 2005).

In epidermal keratinocytes, NGF increases the expression of the proinflammatory cytokine RANTES, known to play an important role in the development of allergic skin infiltrates (Raychaudhuri and Raychaudhuri, 2004) beyond its role in neurogenic inflammation. Moreover, it was shown recently that NGF expression correlates with granularity of eosinophils and that BDNF via binding to TrkB and p75NTR can promote eosinophil survival and migration in atopic individuals (Raap and Kapp, 2005). Last, the expression of NT-4 was increased in prurigo lesions and could be upregulated by IFN-γ injection into the skin (Grewe *et al.*, 2000), linking again inflammatory cytokines with NT expression, although the functional relevance of these observations requires additional confirmation. These data suggest NTs as important players in the neuroimmune network regulating allergic skin response.

NTs and psoriasis. Psoriasis is a common chronic inflammatory skin disease (Raychaudhuri and Raychaudhuri, 2004). Although significant progress has been made in elucidating the pathogenesis of psoriasis, the molecular mechanisms that control the inflammatory and proliferative processes in psoriasis are still under investigation. Cytokines, chemokines, growth factors, adhesion molecules, neuropeptides, and T-cell receptors act in concert to induce unique inflammatory and proliferative processes, typical for psoriasis.

The role of NGF is particularly relevant in the pathogenesis of

psoriasis. Immunohistochemical studies revealed that keratinocytes in lesional and non-lesional psoriatic tissues express high levels of NGF, compared to the controls (Fantini *et al.*, 1995; Raychaudhuri *et al.*, 1998). NGF can influence many pathologic processes, such as proliferation of keratinocytes, angiogenesis, T-cell activation, expression of adhesion molecules, increase of cutaneous innervation, and upregulation of neuropeptides, all known to take place in psoriasis (Raychaudhuri and Raychaudhuri, 2004).

Although clinical and laboratory studies suggest a critical role of NGF and its receptor system in the inflammatory process of psoriasis, direct evidence had been lacking. To determine the significance of the NGF/NGF receptor system in the inflammatory process of psoriasis, the effects of TrkA inhibitor K252a was evaluated. In a double-blinded, placebo-controlled study, the role of NGF/NGF-R in psoriasis was addressed in an *in vivo* system using the severe combined immunodeficient mouse–human skin xenograft model of psoriasis (Raychaudhuri *et al.*, 2004). The transplanted psoriatic plaques on the severe combined immunodeficient mice were treated with K252a, a high-affinity NGF receptor blocker. Psoriasis significantly improved following 2 weeks of therapy and the length of the rete pegs became significantly ($P<0.01$) shorter. A similar improvement in psoriasis was observed by directly antagonizing NGF with NGF-neutralizing antibody.

Also, the effects of NGF on endothelial cell biology were reported. NGF is mitogenic to endothelial cells and induces intercellular adhesion molecule expression on these cells (Raychaudhuri *et al.*, 2001). NGF effects on endothelial cells could be inhibited by K252a- and NGF-neutralizing antibody. To substantiate the effect of NGF on intercellular adhesion molecule expression, the study was carried out using skin explants. Normal human skin samples grafted onto severe combined immunodeficient mice were injected with NGF and upregulation of intercellular adhesion molecule on endothelial cells was noticed within 2 hours of injection of NGF. This induction of intercellular adhesion molecule was inhibited when NGF and K252a were injected simultaneously. However, K252a did not affect the upregulation of intercellular adhesion molecule on endothelial cells induced by substance P (unpublished data).

Taken together, these data suggest that NGF is involved at least in part in the pathophysiological control of psoriasis, and that inhibition of Trk signalling may be beneficial in psoriasis treatment. Moreover, preliminary recent data from the murine system suggest that, *vice versa*, potent proinflammatory cytokines like tumor necrosis factor-α, IL-1, and INF-gamma can upregulate the cutaneous expression of NGF, NT-3, NT-4, and their p75NTR *in vivo* (Bläsing *et al.*, 2005), and may thus contribute to a vicious cycle of proliferative, antiapoptotic, and proinflammatory events that maintain and promote hyperproliferative, inflammatory skin diseases such as atopic dermatitis and psoriasis.

Perspectives

In summary, NTs can influence numerous cellular functions in normal and diseased skin, and NT effects on skin cell fate (survival, apoptosis, and differentiation) are likely to strongly depend on multiple signalling pathways that are activated by NTs in different cells under different conditions. Despite substantial progress in understanding the molecular mechanisms of NT signalling during the last decade, additional efforts are required to fully understand mechanisms controlling the expression patterns of NTs, their receptors and co-receptors, as well as intracellular adaptor molecules in normal and pathologically altered skin cells. Moreover, given the multiple NT target cells in skin (see Figure 2), and the very complex intracutaneous signalling loops between these skin cell populations that NTs feed into and/or are part of (e.g. via the modulation of skin nerve fibers, mast cells, melanocytes, and Merkel cells, another key challenge is to dissect direct from indirect NT-mediated changes in any given cutaneous tissue compartment under physiological and pathological conditions, and to develop adequate pharmacological tools for targeting only the clinically desired elements of NT signalling in the skin. For example, the systemic administration of NGF for the treatment of peripheral neuropathies has to cope with the problem of pain and pruritus induction (see Aloe, 2004), and to be able to dissociate, for example, the antiapoptotic and proliferation-stimulatory effects of NGF on epidermal keratinocyte from its (e.g. mast cell- and substance P-mediated) proinflammatory ones is an important challenge. Progress in these neglected areas of skin and NT research certainly is a prerequisite for the development of satisfactory new treatment modalities for several major pathological skin conditions, such as alopecia, psoriasis, atopic dermatitis, and tumor growth, based on the well-targeted modulation of selected aspects of NT signalling.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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